solution revealed that they regenerated the halted activity.

3.3 Zoosporicides

The methanol extract of unripe fruits of *Ginkgo biloba* L showed lytic activity toward the zoospores and the bioassay-guided fractionation resulted in isolation of a mixture of anacardic acid homologues (**11**) as an active principle.

3.4 Fungitoxins

Antifungal substances against *A. cochlioides* detected on agar plates by a paper disc method, were isolated from roots of *Chelidonium majus* L var *asiaticum* and *Solidago gigantea* (Aiton) var *leiophylla*, and identified as a sanguinarine alkaloid (**12**), inhibitory at 0.25 μg per disc) and a furan-containing diterpene (**13**, inhibitory at 2.5 μg per disc). The compounds are known to exist in *Fumaria indica* Pugsl seeds[5] and *Solidago serotina* (*S gigantea*),[6] respectively, and both inhibited growth of the fungus at 2.5 μg per disc).

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Role of the α subunit of nicotonic acetylcholine receptor in the selective action of imidacloprid

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Abstract: Examination of agonist interactions of imidacloprid on recombinant chicken α4β2 and *Drosophila* SAD/Chicken β2 hybrid receptors, expressed in *Xenopus* oocytes by nuclear injection of the cDNAs, indicates that imidacloprid is a partial agonist. Replacement of the α4 subunit for the *Drosophila* SAD subunit lowered the imidacloprid EC_{50} 37-fold, whereas EC_{50}s for other agonists increased 4-50 fold, suggesting that the α subunit contributes to the high affinity of insect nicotonic receptors for imidacloprid.

Keywords: Imidacloprid; neonicotinoid; epibatidine; chicken α4β2 nicotinic receptor; *Drosophila* SAD subunit

1 INTRODUCTION

Chloronicotinyl insecticides and related nitromethylene heterocycles target insect nicotinic acetylcholine receptors (nAChRs).[1–3]. Electrophysiological and radioligand binding studies have shown these neonicotinoids to be more active against insect than against vertebrate nAChRs.[4,5] The molecular basis of the interactions of these insecticides with nAChRs remains to be determined.

nAChRs are heteropentamers; each molecule is composed of two α and three non-α subunits surrounding a central cation-selective ion channel that opens transiently in response to the binding of ACh or nicotinic agonists. From insect species, six nAChR subunits have been studied in detail, but apart from the αL1 subunit of *Locusta migratoria* L none of subunits from *Drosophila melanogaster* Meig is capable of forming functional receptors in any combinations tested so far in three heterologous expression systems (*Xenopus laevis* oocytes, HEK cells and *Drosophila* S2 cell lines.[6,7] Nevertheless, α subunits ALS, SAD and Dα3 of *Drosophila* are able to form functional receptors when co-expressed with the chicken β2 subunit (Reference 6, and E D Gundelfinger pers. comm.). Using this unique property we examined the sensitivity to imidacloprid of chicken α4β2 receptors and *Drosophila* SAD/chicken β2 hybrid receptors in an attempt to clarify the role of the α subunit in selectivity.

2 MATERIALS AND METHODS

Recombinant nAChRs were expressed in *Xenopus* oocytes by nuclear injection of cDNAs inserted into appropriate expression vectors, typically the pMT3 vector.[8] Briefly, the nucleus of each oocyte was injected with each cDNA (1 ng in 20 nl of distilled water). The injected oocytes were incubated at 17–18°C in standard oocyte saline (SOS): NaCl(100 mM) KCl(2 mM); $CaCl_2$(1.8 mM); $MgCl_2$(1 mM) and HEPES (5 mM; pH 7.5) supplemented with penicillin (100 units ml^{-1}), streptomycin (100 μg ml^{-1}), gentamycin (50 μg ml^{-1}) and sodium pyruvate (2.5 mM). Electrophysiological recordings were performed two to four days after incubation.

Oocytes were secured in an 80-μl Perspex recording chamber. SOS used to bathe oocytes was delivered by a gravity-fed system at a flow rate of 5–7 ml min^{-1}. Atropine (0.5 μM) was added to the saline to suppress endogenous muscarinic responses.

Electrophysiological responses of chicken $\alpha 4\beta 2$ and *Drosophila* SAD/chicken $\beta 2$ hybrid receptors were measured by the two-electrode voltage clamp method. Membrane currents evoked by bath-applied compounds were recorded using a Geneclamp 500 amplifier and digitized data were stored on a computer and analysed with Axotape software (Axon Instruments). Oocytes were challenged with compounds at intervals of 3–5 min to minimise desensitization of the receptors. Membrane potentials were clamped at −100 mV.

3 RESULTS AND DISCUSSION

Imadacloprid, as well as acetylcholine, (−)-nicotine and (+)-epibatidine (Fig. 1) evoked inward currents in *Xenopus* oocytes expressing chicken $\alpha 4\beta 2$ and *Drosophila* SAD/chicken β_2 hybrid receptors (Fig. 2). The pEC_{50} ($\log_{10} EC_{50}$) value of imidacloprid was the lowest (<4.1) while (+)-epibatidine, which shares with imidacloprid the 2-nitroimino-imidazolidine moiety, had the highest pEC_{50} value (8.9) of the ligands tested on the $\alpha 4\beta 2$ receptors. The maximum response of the α4B2 receptor to saturating concentrations of imidacloprid was smaller than that evoked by acetylcholine and thus the former was a partial agonist. By contrast (−)-nicotine and (+)-epibatidine were full agonists of the vertebrate receptor.

Replacement of the α4 subunit for the *Drosophila* SAD subunit lowered the EC_{50} value 37-fold, whereas the EC_{50} values of other agonists tested were increased 4–50 fold.[9] Imidacloprid and (−)-nicotine were partial agonists of the SAD $\beta 2$ receptor, while (+) − epibatidine behaved as a full agonist. These results were not much affected by substituting Ca^{2+} ions for Ba^{2+} ions in SOS, this excluding any distortion of the results due to involvement of Ca^{2+}-activated chloride channels. Our finding that, in the presence of the SAD subunit, the apparent affinity of imidacloprid increased, contrasted sharply with the reduced affinity of (+)-epibatidine which shares the 6-chloro-3-pyridyl moiety with imidacloprid. This demonstrates that the 2-nitromino-imidazolidine moiety of imadacloprid at least partly interacts with the α-subunits and the *Drosophila* subunit is better able to recognize this moiety than the azabicycloheptane moiety of epibatidine.

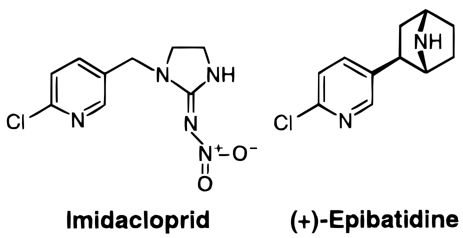

Figure 1. Chemical structures of imidacloprid and (+)-epibatidine.

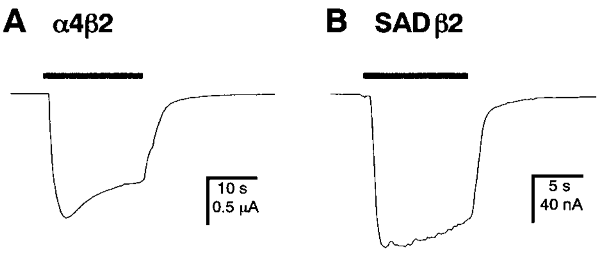

Figure 2. Activation of (A) chicken $\alpha 4\beta 2$ and (*B*) *Drosophila* SAD/chicken $\beta 2$ hybrid receptors by bath-applied imidacloprid. A, inward currents evoked by 100 μM imidacloprid; B, inward current evoked by 4 μM imidacloprid in *Xenopus* oocytes.

Imidacloprid was a partial agonist at both types of recombinant nAChR studied. If such a partial agonist action is the mechanism of action on insect receptors, imidacloprid might compete with acetylcholine, thereby suppressing cholinergic transmission in the insect nervous system. Unlike imidacloprid, (+) − epibatidine, which shares the pyridyl moiety with imadacloprid, was a full agonist at $\alpha 4\beta 2$ and SAD $\beta 2$ receptors, suggesting that the 2-nitroimino-imidazolidine moiety plays a role in the partial agonist action.

Effects of imidacloprid on the ACh-mediated response were also examined at concentrations at which it did not apparently induce inward currents. Imidacloprid augmented the ACh-mediated response of the $\alpha 4\beta 2$ receptor, whereas it slightly suppressed the response of the SAD $\beta 2$ receptor, providing further evidence for interaction of imidacloprid with the α subunit. The latter action can be explained by antagonism of imidacloprid to ACh binding, but the action on the $\alpha 4\beta 2$ receptor is more complex.

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Oxazolidinones: a new chemical class of fungicides and inhibitors of mitochondrial cytochrome bc$_1$ function

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Abstract: Famoxadone is a preventative and curative fungicide recently commercialized for plant-disease control. The molecule and its oxazolidinone analogs are potent inhibitors of mitochondrial ubiquinol : cytochrome c oxidoreductase (cytochrome bc$_1$) and they bind in the Q$_0$ site of the enzyme near the low potential heme of cytochrome b. Inhibitor binding constants for five mutant cytochrome bc$_1$ enzymes from *Saccharomyces cerevisiae* having single amino acid changes in their apocytochrome b located near the low potential heme were compared with their two parental wild-type enzymes. The five individual amino acid changes altered the inhibition constants for the inhibitors famoxadone, myxothiazol, azoxystrobin, and kresoxim-methyl in dissimilar fashion. The log scale differences in binding constants relative to those of their parentals provide fingerprints for the effects of the amino acid changes on binding of the individual inhibitors, thus reflecting the structural diversity of the inhibitors.

Keywords: famoxadone; mode of action; fungicide; plant disease; cytochrome bc$_1$; enzyme inhibitors; oxazolidinones

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1 INTRODUCTION

Famoxadone (3-anilino-5-methyl-5-(4-phenoxyphenyl)-1,3-oxazolidine-2,4-dione; Famoxate®; DPX-JE874) is a newly developed fungicide useful for preventative and curative control of fungal diseases in crops.[1] The fungicidal properties of this molecule were discovered through a chemical scouting and optimization methodology using enzyme and whole-plant data rather than through an analog program against natural products or other known fungicides.[2] From results of independent experimental methods, we have determined that famoxadone and its analogs bind to the Q$_0$ site of cytochrome bc$_1$ and thereby inhibit the enzyme's catalytic functions. Further, we have concluded that inhibition of cytochrome bc$_1$ function is the primary cause of famoxadone's physiological and fungicidal properties. In this report we summarize studies which indicate that famoxadone has a different binding mode to the enzyme than other inhibitors of cytochrome bc$_1$, which are also known to bind to the enzyme's Q$_0$ site.

2 EXPERIMENTAL

Saccharomyces cerevisiae Meyer ex Hansen parentals and their isolates having single amino acid changes in their apocytochrome b were a kind gift from Dr A-M Colson and they have been described in detail elsewhere.[3] All other experimental details will be presented elsewhere.

3 RESULTS AND DISCUSSION

Submitochondria were isolated from two wild-type parentals of *S. cerevisiae* and five of their isolates, which have been characterized as having single amino acid changes in their apocytochrome b.[3] IC$_{50}$ values of four Q$_0$ center inhibitors of mitochondrial cytochrome bc$_1$, famoxadone,[1,2] azoxystrobin,[4,5] kresoxim-methyl[6] and myxothiazol[7] (Fig. 1), were determined against each of the mitochondrial preparations by measuring inhibition of the overall reaction NADH to O$_2$ by following the oxidation of NADH spectrophotometrically. The IC$_{50}$ values of the five isolates, compared to those of their respective wild-type parentals, gave the relative values shown in log scale in Fig 1 for the individual inhibitors.

Inspection of the fingerprint profiles presented in Fig. 1 indicates considerable differences between the four Q$_0$ center inhibitors with respect to the influences of individual amino acid changes in apocytochrome b and their relative IC$_{50}$ values. The G137R, L275S, and F129L changes were detrimental to the binding of all four inhibitors by factors of 3.8–180, 1.5–24, ad 2.2–64, respectively. The L275F change enhanced the binding of famoxadone by a factor of 20 and that of azoxystrobin by a factor of 2.5, whereas the L275F change decreased the affinity for kresoxim-methyl by a factor of 1.8 and that of